Isolation and characterization of polysaccharides from *Fraxinus angustifolia* infusions

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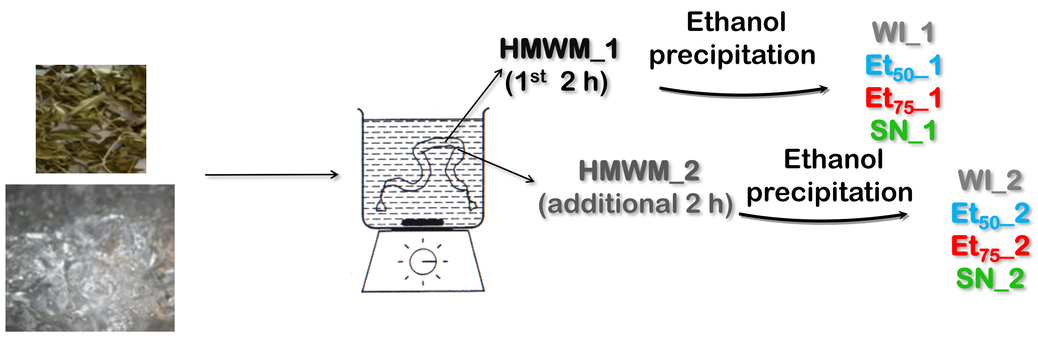
Introduction

In the Trás-os-Montes region, the use of infusions of *Fraxinus angustifolia* dried leaves as a protection against high levels of cholesterol, blood pressure and uric acid is widespread [1]. Pectic polysaccharides isolated from the infusions of some medicinal plants have been reported as biologically active [2]. Pectic polysaccharides have been described as structurally complex polymers, exhibiting different polymeric building blocks: homogalacturonans (HG), rhamnogalacturonans-I (RG-I), rhamnogalacturonans-II (RG-II) and xylogalacturonans (XG) [3]. The backbone of RG-I can be partly substituted with various side chains, such as arabinans, type-I and type-II arabinogalactans (AG-I and AG-II). The aim of this work is to provide a insight regarding the nature of the pectic polysaccharides present in the infusions of *F. angustifolia* dried leaves.

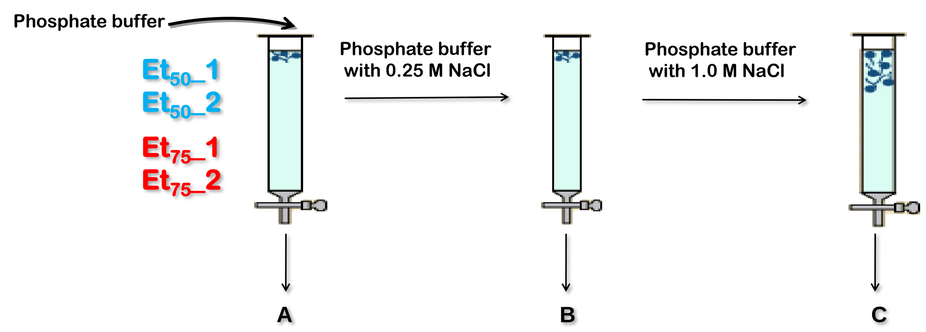
Methodologies

I- Preparation of the High Molecular Weight Material (HMWM) and Ethanol Precipitation

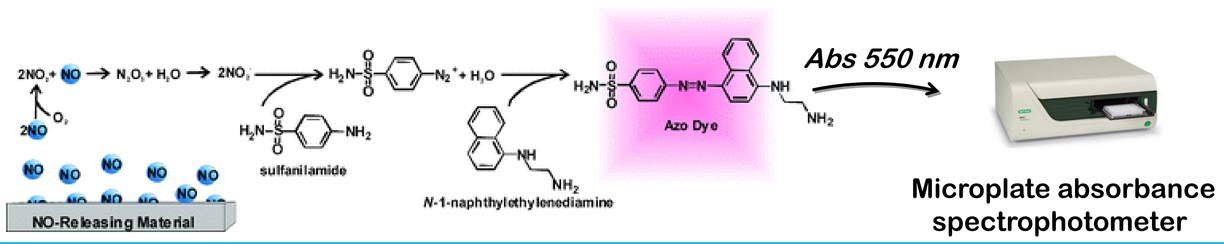


Fraxinus angustifolia dried leaves in boiling water (0.05-0.07 mg/mL) during a total of 4h (2 h + 2 h)

II- Anion Exchange Chromatography (DEAE-Sepharose FF AEC)



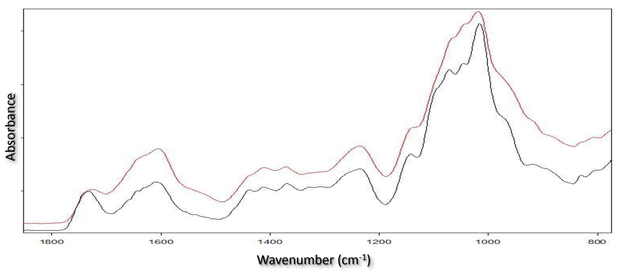
III- NO production by Griess reagent



Microplate absorbance spectrophotometer

IV- FTIR analysis

- 8 cm^{-1} resolution
- 128 co-added scans
- Absorbance mode
- 4000-550 cm^{-1}



Results

Table I- Glycosidic content and monosaccharide composition of the HMWM´s and various fractions obtained by ethanol precipitation.

	Glycosidic content (mass%)	Monosaccharide Composition (mol %)						
		Rha	Ara	Xyl	Man	Gal	Glc	UA
1st 2 h	57.0	1.8	5.3	0.9	3.0	8.6	9.4	71.1
Et_{50}	81.0	2.1	3.5	1.6	0.5	3.2	3.0	86.3
Et_{75}	55.8	3.4	11.2	2.6	2.6	12.8	9.8	57.8
SN	33.0	8.4	17.0	1.2	11.0	5.3	33.9	23.3
2nd 2 h	76.1	1.6	6.8	0.8	1.7	8.1	4.8	76.4
Et_{50}	90.1	1.6	4.3	1.5	0.2	2.9	1.1	88.5
Et_{75}	84.7	2.5	10.6	2.6	1.1	9.5	4.1	69.8
SN	38.8	5.3	35.7	1.2	8.0	4.5	22.3	23.1

Et_{50}

Methylation analysis

❑ Pectic polysaccharides "enriched" in HG domains

Et_{75}

❑ Pectic polysaccharides "enriched" in RG domains

Figure 1- Tentative structures for the arabinan and galactan moieties present in the pectic polysaccharides "enriched" in HG domains.

Table II- Yield, glycosidic content and monosaccharide composition of various fractions obtained by anion exchange chromatography.

	Yield (mass %)	Glycosidic Content (mass%)	Monosaccharide Composition (mol %)						
			Rha	Ara	Xyl	Man	Gal	Glc	UA
Et_{75} 1st 2 h									
A	54.3	85.9	0.9	7.9	3.9	3.7	10.8	5.7	67.1
B	31.2	55.8	5.7	17.6	1.7	0.3	13.5	2.8	58.2
C	14.5	13.9	17.9	5.4	0.6	1.4	5.9	43.8	25.0

❑ Retained fraction B exhibits a lower UA proportion

FTIR analysis

❑ Retained fraction B exhibits a higher neutral sugar proportion

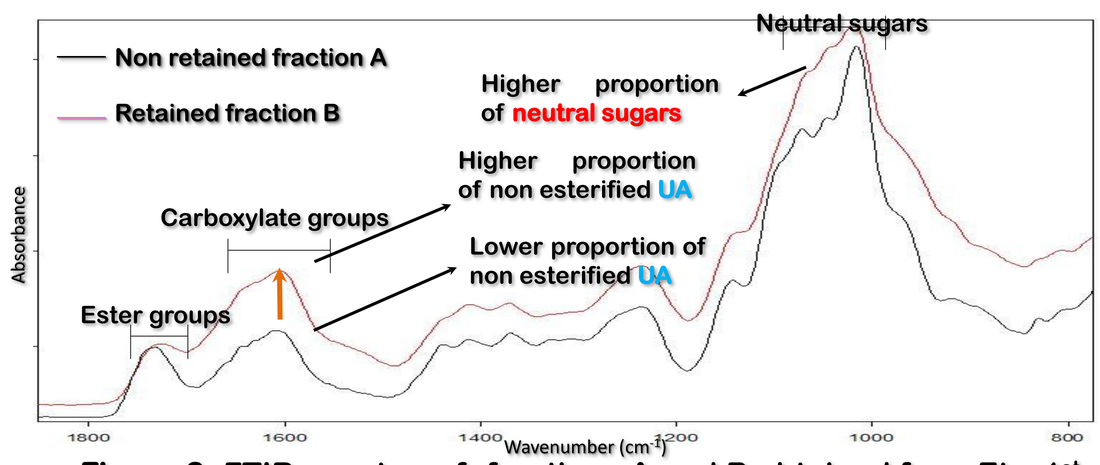


Figure 2- FTIR spectra of fractions A and B obtained from Et_{75} 1st 2h by DEAE-Sepharose anion exchange chromatography.

NO production

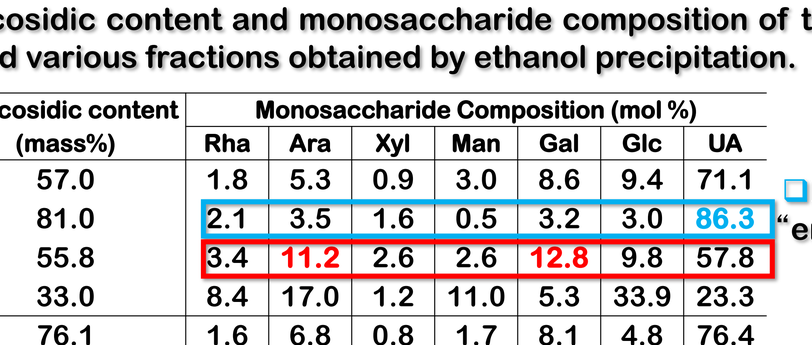


Figure 3- NO production determined by Griess reagent for **A** (128 µg/mL) and **B** (170 µg/mL) fractions obtained from Et_{75} 1st 2 h.

Potential pro--inflammatory effect

❑ Fractions A and B obtained from Et_{75} 1st 2 h should present AG-II regions like the ones presented in **Figure 1**, which are similar to structures reported as biologically active [4].

Conclusions

- Infusions from *Fraxinus angustifolia* contained high molecular weight material comprising a mixture of polysaccharides, including pectic polysaccharides.
- Ethanol precipitation allowed to fractionate pectic polysaccharides according to uronic acid content, possibly related to the proportion of HG and RG-I regions present in their composition.
- Besides the UA content of the material, its degree of methylesterification also seems to be a feature that contributed to the fractionation by DEAE-Sepharose anion exchange chromatography.
- The biological activity displayed by fractions A and B, obtained from Et_{75} 1st 2 h, seems to be related to the higher proportion of neutral sugars, organized in AG-II regions already reported as biologically active.

References

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